Developing New High Yielding Rapeseed Variety

Development of short duration higher yielding variety in *Brassica rapa* is an important task to meet the demand of the farmers of the country who want to grow three crops in a year. One mustard crop can be grown in a Aman-Rapeseed-Boro cropping pattern if a rapeseed variety of at least 80 days maturity period with higher yield potential can be developed. Generation of higher yielding short duration rapeseed / mustard genotypes to utilize them in the variety development programme is one of the highest research priority agenda to meet the national demand and to cut down the increasing edible oil import.

With this end in view, a research sub-project was undertaken with financial assistance from PIU-BARC of NATP and is being implemented by Sher-e-Bangla Agricultural University for a period of three years.

Field view of the F_2 population of rapeseed

Approach and Methodology

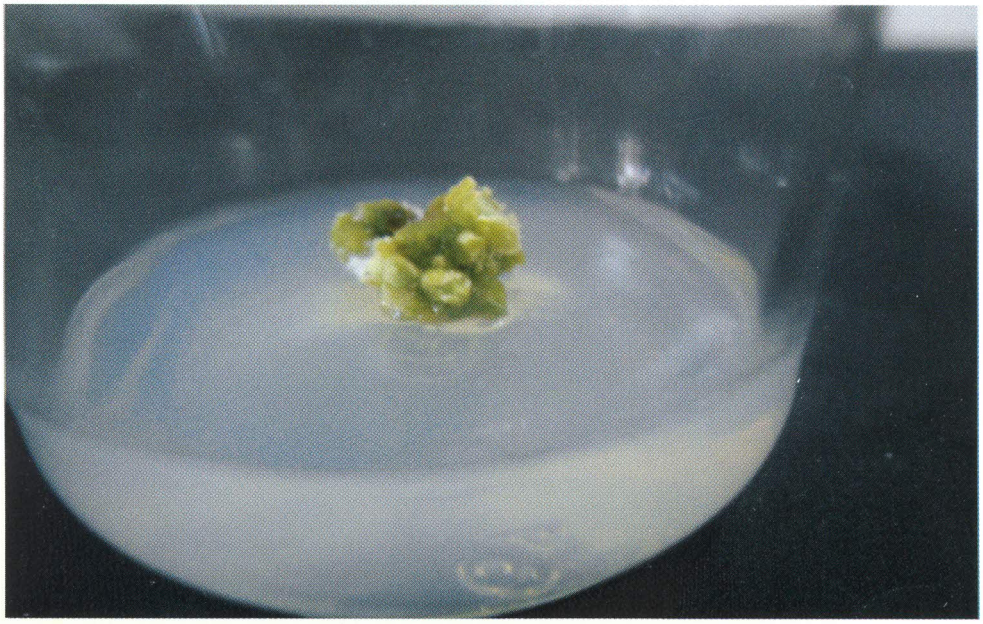
Production of huge number of variability in homozygous state in a short period of time following pollen / anther culture and artificial chromosome doubling to allow selection in stable materials is now becoming a breeding strategy of *Brassica* oilseed crops. This strategy is used almost routinely in some countries in two tetraploid species of oleiferous *Brassica* - *Brassica napus* and *B. juncea* and to carry out effective selection programme. Our main oil crop is rapeseed, a diploid species *B. rapa*. The little pollen/anther culture research carried out with this diploid rapeseed species did not give much positive result. It was thus the main aim of this sub-project to develop reliable working protocol to generate variations through culture of anther / pollen of *B. rapa* in order to develop short duration higher yielding yellow seeded rapeseed genotypes which can fit well into Aman – Rapeseed – Boro cropping pattern.

The characteristics of six parental materials of rapeseed used for crossing to produce F_1 seeds are given in Table 1. The field experiments with the F_1s and their successive generations were carried out in the farm of Sher-e-Bangla Agricultural University, Dhaka during 2010-11 to 2011-12. The initial activity of anther culture and pollen culture has been started since rabi 2010 following the protocol used in

Table-1. Main features of the six genotypes used in crossing programme

Genotypes	Days to maturity	Seed coat color	Yield (kg/ha)
SAU Sarisha 1	80-90	Yellow	1200
SAU Sarisha 2	85-95	Yellow	1300
SAU Promising 3	80-90	Brown	1300
BARI Sarisha 6	100-110	Yellow	1800
BARI Sarisha 15	80-90	Brown	1300
Tori 7	70-80	Brown	1000

tetraploid species- *B. napus* and *B. juncea*. The anther / pollen of *B. rapa* did not respond well to the protocol developed for tetraploid species. The pollens of the *B. rapa* did not respond at all to produce any embryos following protocol developed for the tetraploid species. No plantlet was obtained through pollen culture. The tissue culture part of the research was carried out at the laboratory of the Department of Biotechnology of Sher-e-Bangla Agricultural University, Dhaka. Thus initiatives were taken to produce plantlets through anther culture with several combinations of growth hormones.



Induction of calli from cultured anther

The first media used were callus induction media and the second media used was for the development of shoot. The third media was for the induction of the root of the already developed shoot and the last media were used for the development of the plantlets. Four cross combinations viz. Tori 7 x SAU sarisha 1, Tori 7 x SAU promising 3, Tori 7 x BARI sarisha 6 and Tori 7 x BARI sarisha 15 showed little but variable response to anther culture. Highest percentage of callus formed through anther culture was found in Tori 7 x BARI sarisha 15 which was 21.55%. Only 10.37% anther formed callus in Tori 7 x BARI sarisha 6. High percentage of calli formed shoot while a very limited number of shoots developed only a few short roots. Ultimately the anther culture did produce only a few plants but did not produce any flowers and seeds as the plants were obtained in February which was not suitable time for flowering and fruiting.

Emergence of shoot from the callus

The F_2 seeds were sown in 2011-12 rabi season along with F_1 remnant seeds kept in refrigerator. Anther culture was carried out with anthers of the F_1 plants of the cross combinations- Tori 7 x SAU sarisha 1, Tori 7 x SAU promising 3, Tori 7 x BARI sarisha 6 and Tori 7 x BARI sarisha 15. To induce callus, hormones of both auxin and cytokinin groups were used in different concentrations. For shoot and root generation, different combinations of hormones with different concentrations were used. Both NLN and MS media were used for anther/pollen culture. Various combinations of hormones were used to induce root in the already regenerated shoot. Comparatively better response was found and some of the regenerated shoots developed quite a good number of long roots. A few small plants were transferred to earthen pots.

However, the F_2 seeds of the fifteen cross combinations were grown in an experiment with three replications in RCBD design. Forty nine F_2 plants were initially selected morphologically from F_2 populations of eleven cross combinations. Further strict selection based on shorter maturity period and overall good per plant yield allowed collection of seed from nineteen F_2 plants of six cross combinations. Eight selected plants showed their maturity period between 73-75 days with individual seed weight per plant ranging between 4.60 to 5.40 g of seed.

It required much time for the development and standardization of repeatable protocol for the anther culture. It was thus late to obtain plantlets through anther culture and as a result these could not be utilized for the production of seed in 2011-12. The lack of green house facility is a limiting factor in providing off-season growth facility to the plants regenerated through tissue culture. To obtain plants that would produce flowers and seeds, the anther culture should be started at the early October to provide plants the flowering period in November or in early December to allow seed production by the regenerated plants.

The methodology developed using various hormonal treatment combinations for the regeneration of homozygous but variable types of plants from anther culture of F_1 and F_2 plants of rape seed could be utilized by the rapeseed breeders in *B. rapa* breeding in future. The successful use of this methodology would allow selection of desired materials from the stable plant materials within a very short period of time as breeding cycles could be shortened by 4-5 generations through anther culture.

Achievements

- Enough hybrid seeds were obtained through intergenotypic crosses
- Repeatable anther culture protocol was established
- Good number of F_2 populations of all the cross combinations were obtained
- Selection of desired plants has already been started in the segregating population

Lessons Learned

- The species *Brassica rapa* showed low response to pollen and anther culture
- Supplementation of accurate combinations of growth hormones is important for anther culture of *B. rapa*
- Green house facility is essential for the improvement of rapeseed through tissue culture

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